



# Effect of Captopril on Inflammatory Biomarkers, Oxidative Stress Parameters and Histological Outcome in Experimental Induced Colitis

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## Abstract

Inflammatory bowel disease of unknown etiology and remain chronic progressive inflammation with no effective line of treatment .It is mandatory to investigate new drugs with more therapeutic efficacy. The aim of the current study is to investigate the effect of Captopril on inflammatory biomarkers , oxidative stress parameters and histological outcome in experimentally induced colitis. Experimental colitis was induced in rats by rectal administration of 4% acetic acid (vol/vol) .Rats with colitis were received either captopril 30mg/kg or sulfasalazine 100mg/kg orally for 7days.Macroscopical and microscopical assessment and the measurement of the colonic cytokines (IL-6 and TNF- $\alpha$ ) , oxidative stress markers ; myeloperoxidase (MPO) and malondialdehyde (MDA) , and adhesion molecules (E-Selectin and ICAM-1) .Our results had shown that both macroscopical lesion area and histological colonic injury induced by acetic acid were significantly reduced by both captopril and sulfasalazine .These were accompanied by attenuation of the elevated colonic MPO activity , MDA and proinflammatory cytokines .Besides downregulation of the adhesion molecules .These results demonstrated that captopril possesses therapeutic potential in experimental colitis . The anti-inflammatory actions involve antioxidant effect along with inhibition of adhesion molecule synthesis in the colonic tissues.

**Key Word** : Acetic acid , Captopril, Oxidative stress ,Adhesion molecules, Ulcerative colitis

## INTRODUCTION

Inflammatory bowel disease (IBD) comprises those conditions characterized by a tendency for chronic and spontaneously relapsing inflammatory disease of the intestines[1]. It is characterized by colon tissue edema and increased epithelial permeability of colon and extensive leukocytes infiltration of in the colon[2]. However, little is known about etiology of this diseases, it is believed to involve an abnormal host response to endogenous or environmental antigens or microbes have been suggested as an important factors for initial tissue injury followed by amplification of this response[3]. There is an evidence for intense local immune response associated with inflammatory cells infiltration giving rise to mucosal disruption and ulceration[4] Activation of these infiltrating cells results in the release of different pro-inflammatory mediators and adhesion molecules that play a crucial role in tissue destruction and propagation of the inflammatory response[5]. Captopril dramatically reduced the expression of intercellular adhesion molecules indicating it is potential protective effect on endothelial damage[6]. Therefore, it was thought worthwhile to study the inductive remission of this drug against colitis. Although there is a report of using angiotensin converting enzyme inhibitors in animal model as prophylactic effect against ulcerative colitis[7], however we will exploit the therapeutic role of captopril in ulcerative colitis. Captopril and other ACE-inhibit the converting enzyme peptidyl dipeptidase that hydrolyzes

angiotensin I to angiotensin II and inactive bradykinin which act in part by stimulate the release of NO and prostaglandins[8]. Moreover these group of drugs have the ability to block rennin –angiotensin –system (RAS) that mediates multiple biological functions including cell growth , inflammation , and fibrosis contributing to the progression of tissue damage[9].

On the other hand it was demonstrated that fibrogenic response to injury is mediated through Angiotensin II induction of TGF- $\beta$ 1 expression[10].

So that blockade of Angiotensin II by ACE-inhibitors or Angitensin II receptor blockers reduces fibrosis through the inhibition of TGF- $\beta$ 1[11] From these finding it has been demonstrated that neutralized of angiotensin II may be beneficial therapeutic target through inhibition of this fibrogenic cytokine in colitis[12].

Captopril a thiol (SH) group containing ACEI dramatically reduces the expression of ICAM-1 that expressed on the surface of endothelial cells of GIT indicating it is potentially protective effect on endothelial damage[6].

## MATERIALS AND METHODS

### Materials

Animals : Adult male albino rats (200-220g) were purchased from animal house of the national center for drug control and researches (NCDCR) . Animal were housed five per cage for one week prior to the experiment and had access to laboratory chow pellet and were allowed

to drink tap water ad libitum. All animal experiments were performed after getting prior approval from the institutional animal ethics committee college of medicine Al-Nahrain university.

Drugs : captopril and sulfasalazine were purchased from Sigma –Aldrich company .

### Experimental Design

This study was conducted on 40 adult male albino –wister rats weighing 200-220g previously submitted to starvation for at least 24hrs . Animals were divided into four group (n=10/group) .Group I kept as control and received no treatment .Group II ,III, IV were subjected to the induction of colitis by rectal administration of 4% acetic acid (AA) (v/v) . Thirty minute after the induction of colitis group II was given normal saline orally ; group III and IV were treated orally with captopril 30mg/kg and sulfasalazine 100mg/kg respectively for 7 days.

### Induction of colonic inflammation

Since prior feeding has been shown to prevent the ulcerogenic action of certain drugs and chemical[13] . Rats were starved for at least 24hrs before the induction of colitis but were be allowed free access to tap water , during starvation , rats were kept in cages provided with a wide wire –mesh floor to avoid coprophagy. On the day of the experiment , water was held two hours before the procedure .

Experimental ulceration in colon tissue was done according to the method described by Mousavizadeh *et al* [14]with slight modification. In brief, under light ether anesthesia rats were administered 5ml/kg of 4% acetic acid (AA) solution (BDH Chemical Ltd., England) by transrectally using a flexible silicone plastic tube with an external diameter of 2mm was inserted rectally into the colon to 8cm . After acetic acid administration, rats were holed horizontally for 2 min to prevent AA leakage. Control animals underwent the same procedure using equal volume of normal saline instead of AA solution.

### Preparation of drugs

All drugs were freshly prepared before administration on the day of the experiment .

Investigated drug (captopril) and the standard sulfasalazine were prepared as suspensions in distilled water using sodium Carboxymethyl cellulose (s CMC) 0.3% W/V .The doses of captopril (30mg/kg) was selected based on other studies reporting cytokine suppressing effect of this drug at this dose[11,15]. Sulfasalazine was used as standard therapy in a dose of 100mg/kg[16] .

### Assessment of colitis

After the end of experiment , animals were sacrificed by an over dose of diethyl ether inhalation and then the abdomen was rapidly dissected and open and the colon was removed .The pieces of colons were cut open in an ice bath cleansed gently using normal saline , and observed normally for macroscopic and microscopic assessment. Then samples

were cut into two pieces , one piece for histopathologic assessment (maintained in neutral formalin 10% as a fixater ) and one piece for immunohistochemistry study

### Macroscopic evaluation

**Colonic mucosal damage** (mean area of colonic mucosal damage)

The excised colonic segment (8 cm proximal to anus ) was immediately immersed in normal saline , cleaned from adherent tissues and then opened longitudinal and rinsed with 0.9% sodium chloride solution to discard the fecal materials .

Then the segment was fixed with pins on a dissecting board , and the area of mucosal damage was measured using a computerized planimeter in accordance to the method described earlier[17].

**Colon edema** The colon specimen of each animal was incised along its mesenteric border and gently washed .This is measured through colon weight (CW). It was used as a index of tissue edema , which reflected the severity of colitis[18].

### Disease activity index (DAI)

To quantify the clinical evaluation of the disease we used the DAI described by Meerveld and Tyler[19] that based which include body weight loss stool consistency , rectal bleeding (gross or occult ) we used five grades of weight loss {0 , no loss or weight gain ; 1 , 1-5 % loss ; 2, 6-10%loss ; 3 : 11-15% loss ; 4: greater than 15% loss } , three grades of stool consistency {0: Normal ; 2 : loose ; 4 : diarrhea } , and three grades of bleeding {0 : normal ; 2 occult blood –positive ; 4 : gross bleeding }.

The presence of occult blood in faces was determine using benzidine test.

The total score of DAI was calculated as combined of these scores divided by 3[20].

### Macroscopic colonic score

The macroscopic colonic score was assessed by the scoring system adapted from[21] as following: score are assigned based on the clinical features of the colon using a scale ranging from 0-4 as follows : 1, intact epithelium with no damage ; 2 , patch type superficial hyperemia ; 3 , generalized patch type hyperemic regions; 4 , generalized hyperemic and hemorrhage.

### Histological evaluations:

The colonic samples were fixed in 10% formalin, dehydrated, embedded in paraffin, deparaffinized with xylene , cut into 4  $\mu$ m sections and stained by Hematoxylin and eosin (H&E). Slides were examined and scored for histopathological evaluation. The slides were coded to prevent observer bias during evaluation. All tissue sections were examined in a blinded fashion by experienced histopathologist and results scored according to Cooper *et al*[22].

**Table 1 :Histopathological score of colitis**

Score	Destruction of epithelium and/ or glandular crypts	Dilation of glandular crypts	Depletion and loss of goblet cells	Inflammatory cell infiltration	Edema	Hemorrhagic mucosa	Crypt abscesses	Apoptosis	Dysplasia
0	morphologically normal	Normal aspect	Normal aspect	absence of infiltration	Absent	Absent	Absent	absent	absent
1	focal destruction	Focal dilation	slightly depleted goblet cells	infiltrate at the sub-epithelial and lamina propria level or crypt bases infiltration	Focal	Focal	focal	Focal	Focal
2	zonal destruction	Zonal dilation	zonally or moderately depleted goblet cells	infiltration reaching muscularis mucosa	zonal and/or moderately diffuse	Zonal	Zonal	Zonal	Zonal
3	diffuse and/or mucosal ulceration involving submucosa and/or diffuse crypt loss	diffusely dilated crypts	diffusely or complete depletion of goblet cells	severe and extensive infiltration reaching submucosa and/or involving muscularis propria	extensive and severe	Diffuse	Diffuse	Diffuse	diffuse

**Table 2: Gross features in control and study groups**

Variable †	Control n=10	Colitis n=10	Capto n=10	Sulfaz n=10
MD(mm <sup>2</sup> )	0.00 ±0.00 C	16.11 ±0.74 A	4.04 ±0.44 B	5.26 ±0.43 B
CW (g)	1.13 ±0.17 C	3.12 ±0.24 A	1.63 ±0.23 B	1.63 ±0.23 B
DAI	0.00 ±0.00 C	10.50 ±1.50 A	1.80 ±0.40 B	2.10 ±0.30 B
MAC score	0.00 ±0.00 D	9.30 ±0.64 A	1.60 ±0.49 C	2.50 ±0.50 B

Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; Capto; captopril; Sulfaz: sulfasalazine; † values expressed as mean ±Standard deviation (SD).

**Table 3 .Histopathological score and adhesion molecules**

Variable †	Control n=10	Colitis n=10	Capto n=10	Sulfaz n=10
Histo score	0.00 ±0.00 D	3.00 ±0.24 A	0.80 ±0.20 C	1.80 ±0.40 B
ICAM-1	0.90 ±0.30 D	4.00 ±0.34 A	1.40 ±0.49 C	2.90 ±0.30 B
CD62	0.90 ±0.30 C	3.90 ±0.30 A	1.80 ±0.40 B	1.90 ±0.30 B

Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference . Capto; captopril; Sulfaz: sulfasalazine; † values expressed as mean ±Standard deviation (SD).

**Table 4: Cytokines and oxidative stress markers immunohistochemical score**

Variable †	Control n=10	Colitis n=10	Capto n=10	Sulfaz n=10
TNF- $\alpha$	0.40 ±0.09 D	4.00 ±0.30 A	1.20 ±0.40 C	2.70 ±0.46 B
IL-6	1.00 ±0.23 D	4.00 ±0.30 A	1.80 ±0.40 C	2.50 ±0.50 B
MDA	1.70 ±0.46 C	4.00 ±0.35 A	2.60 ±0.49 B	2.70 ±0.46 B
MPO	0.80 ±0.40 D	4.00 ±0.45 A	1.10 ±0.30 C	1.70 ±0.46 B

Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; Capto; captopril; Sulfaz: sulfasalazine; † values expressed as mean ±Standard deviation (SD).

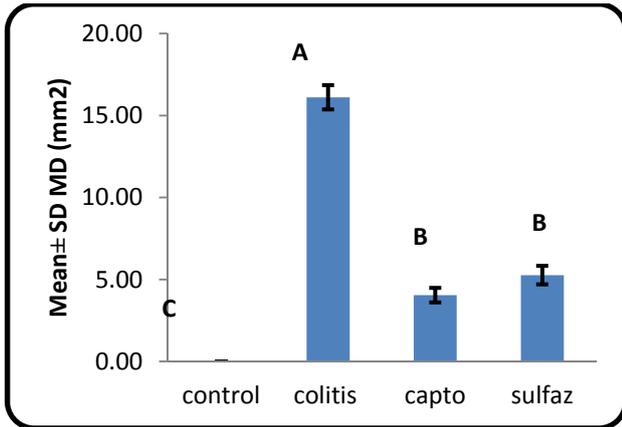


Figure 1: Mean area of mucosal damage in control and study groups

Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; MD : Mucosal damage ; capto; captopril; Sulfaz: sulfasalazine

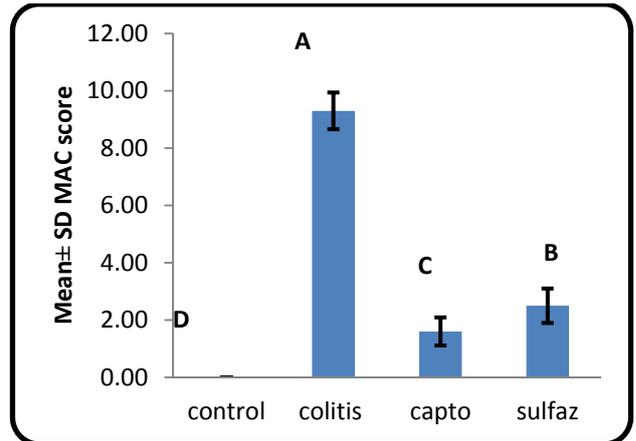


Figure 4: Mean macroscopic score (MAC) in control and study groups

Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; capto; captopril; Sulfaz: sulfasalazine

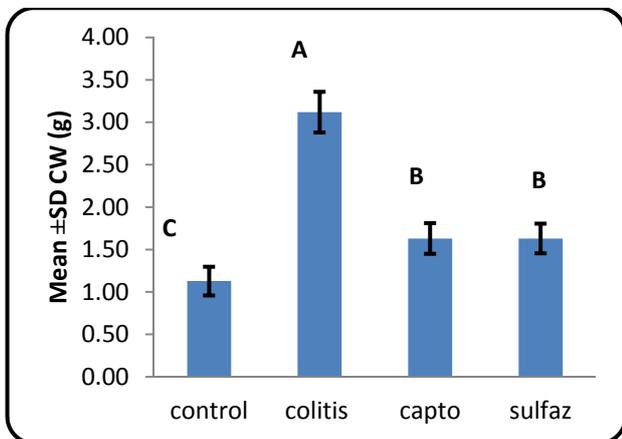


Figure 2: Mean colonic weight (CW) in gram in control and study groups

Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; capto; captopril; Sulfaz: sulfasalazine

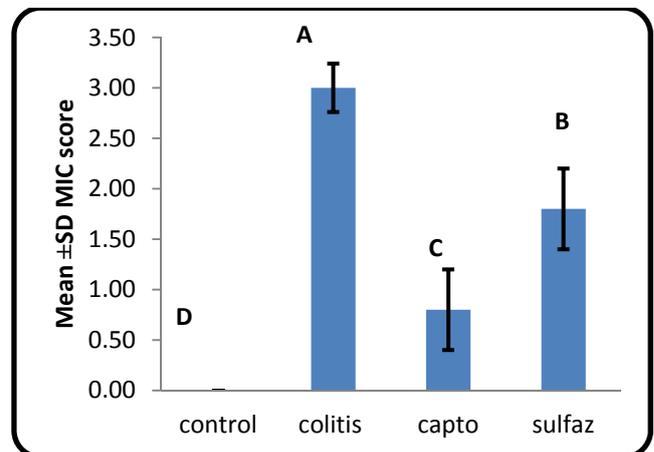


Figure 5: Mean histopathological score (MIC) in control and study groups Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; capto; captopril Sulfaz: sulfasalazine

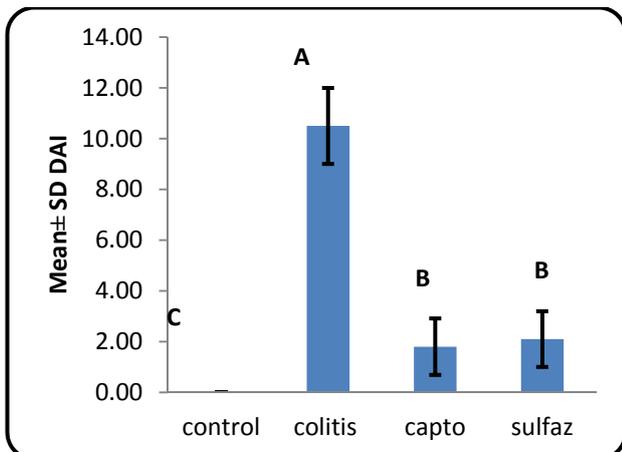


Figure 3: Mean disease activity index (DAI) in control and study groups

Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; capto; captopril; Sulfaz: sulfasalazine

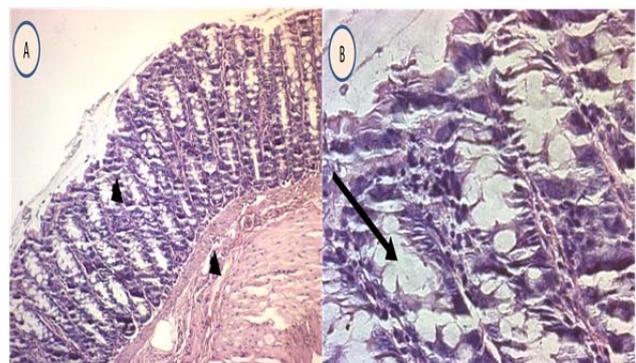
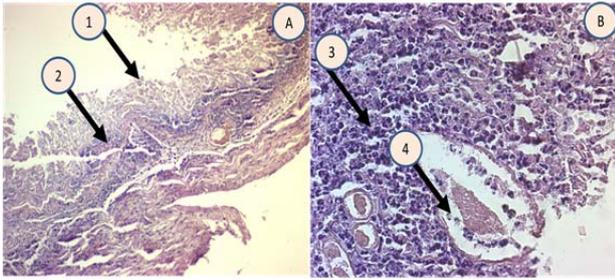
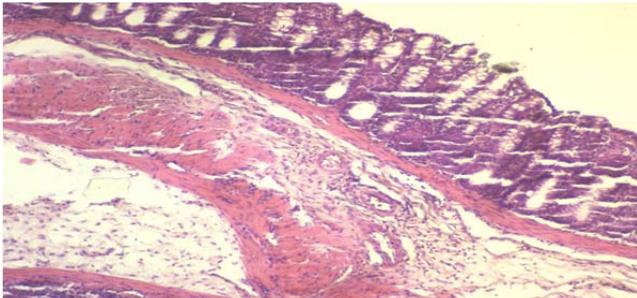


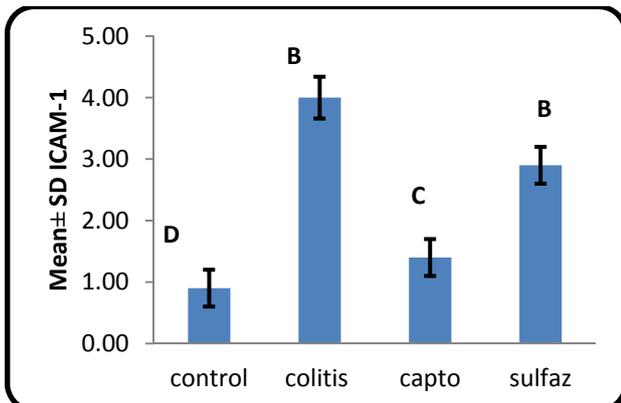
Figure 6: Histological section through colonic wall showing normal mucosal and submucosal pattern with no evidence of inflammation (arrow head) and preservation of goblet cells (arrow); A: 10X; B: 40 X; H and E stain.



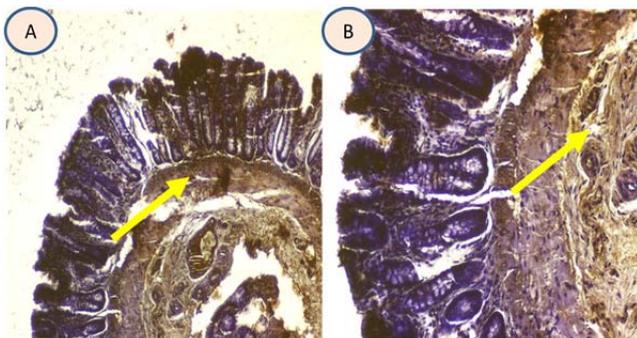
**Figure 7:** Histological section through colonic wall showing mucosal ulceration (1); superficial inflammation (2); mononuclear inflammatory infiltrate (3) and crypt abscess (4) in experimentally induced colitis in rat; A: 10X; B: 40 X; H and E stain.



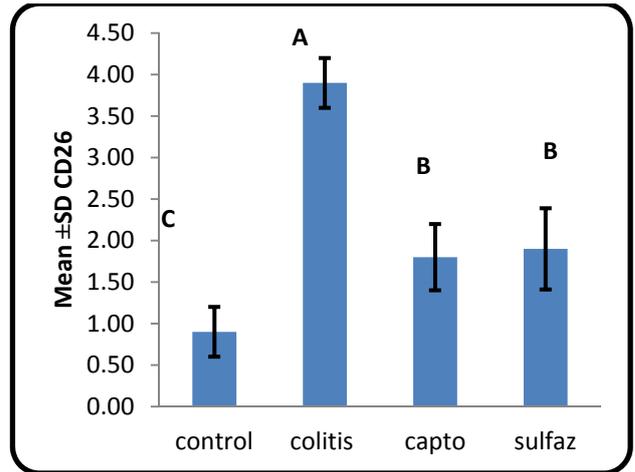
**Figure 8:** Histological section through colonic wall showing drug effects in which there is evidence of mucosal regeneration and glandular formation, less severe inflammation, and goblet cells regeneration (3); 10X; H and E stain



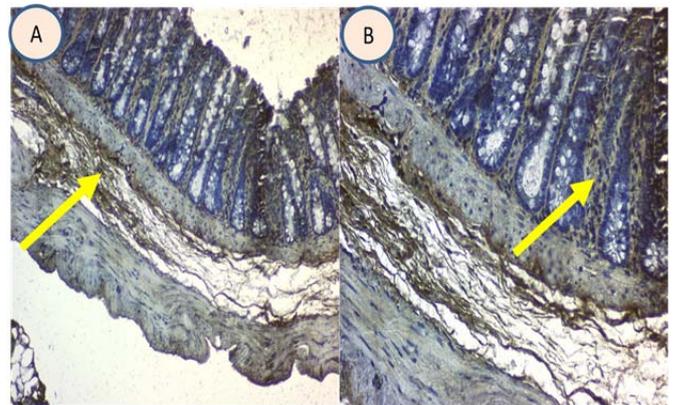
**Figure 9:** Mean intracellular adhesion molecule-1 (ICAM-1) score in control and study groups  
Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; capto; captopril; Sulfaz: sulfasalazine



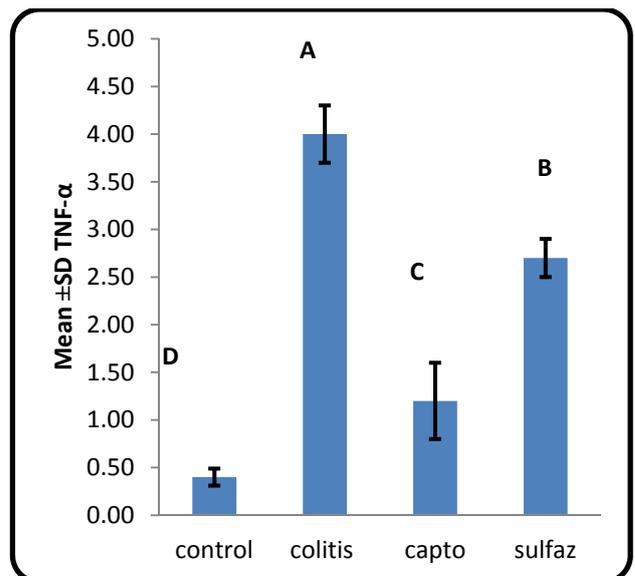
**Figure 10:** Immunohistochemical expression of ICAM-1 showing membranous pattern (yellow arrow); A: 10X; B: 20X.



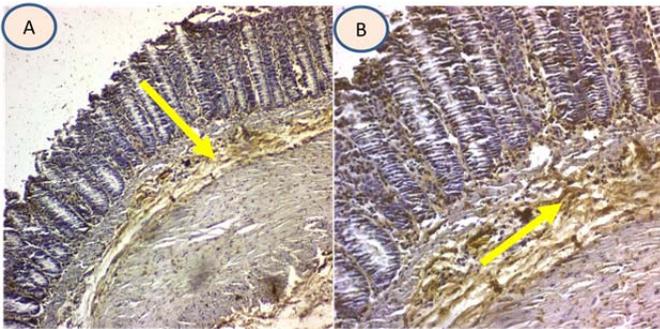
**Figure 11:** Mean CD62 score in control and study groups  
Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; capto; captopril; Sulfaz: sulfasalazine



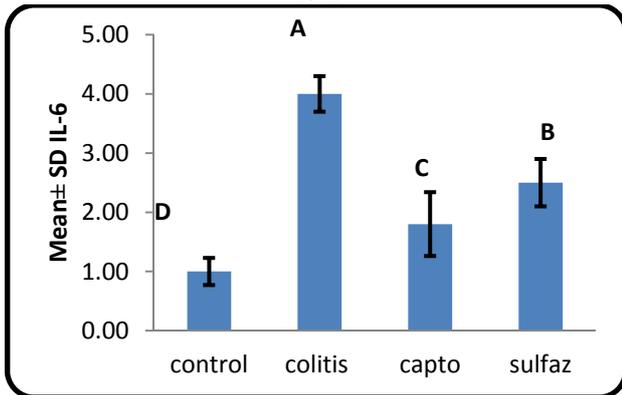
**Figure 12:** Immunohistochemical expression of CD 62 showing membranous pattern (yellow arrow); A: 10X; B: 20X.



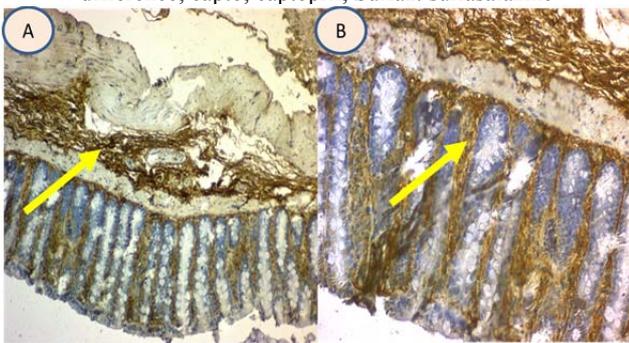
**Figure 13:** Mean TNF-α score in control and study groups  
Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; capto; captopril; Sulfaz: sulfasalazine



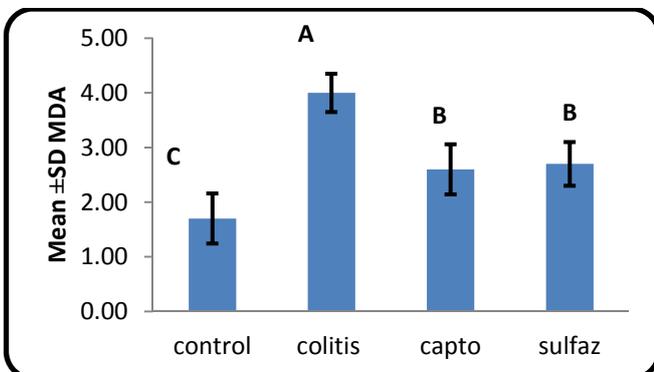
**Figure 14:**Immunohistocheical expression of TNF- $\alpha$  showing membranous and secretory pattern (yellow arrow); A: 10X; B: 20X.



**Figure 15:** Mean IL-6 score in control and study groups Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; capto; captopril; Sulfaz: sulfasalazine

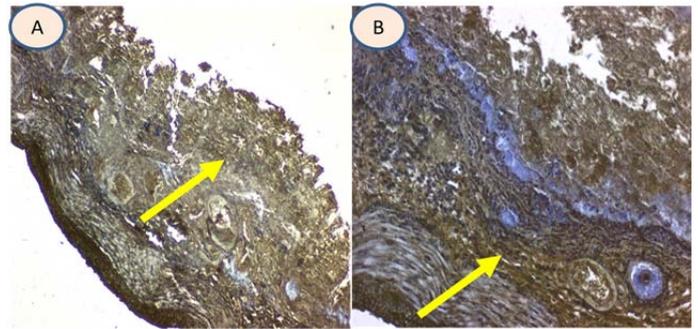


**Figure 16:**Immunohistocheical expression of IL-6 showing secretory pattern (yellow arrow); A: 10X; B: 20X.

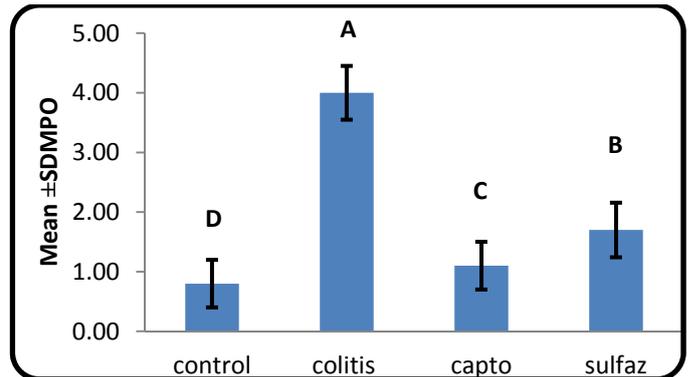


**Figure 17:** Mean malondialdehyde (MDA) score in control and study groups

Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; capto; captopril; Sulfaz: sulfasalazine

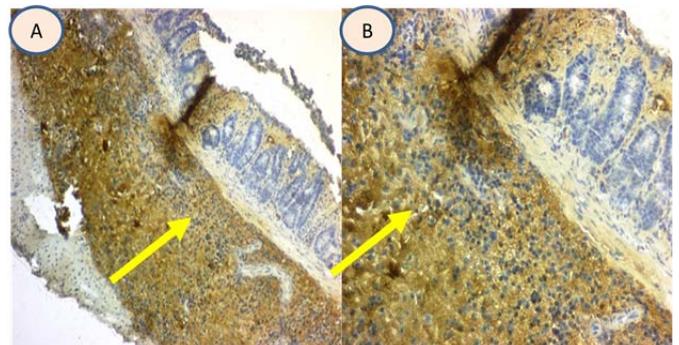


**Figure 18:**Immunohistocheical expression of MDA showing cytoplasmic pattern (yellow arrow); A: 10X; B: 20X.



**Figure 19:** Mean myeloperoxidase (MPO) score in control and study groups

Capital letters for comparison; different letters indicates significant difference; similar letters indicates insignificant difference; capto; captopril; Sulfaz: sulfasalazine



**Figure 20:**Immunohistocheical expression of MPO showing cytoplasmic pattern (yellow arrow); A: 10X; B: 20X

### Immunohistochemistry

Immunohistochemistry offers the advantage of directly demonstrating cells in the affected tissue[23]. The advent of specific antibodies developed for immunohistochemical reactions, together with the standardization of a specific method to meet the objectives of the present study, permitted analysis of the production of various biochemical markers in the paraffin-embedded intestine samples for measurement of the colonic cytokines (IL-6 and TNF- $\alpha$ ), oxidative stress markers ( myeloperoxidase (MPO) and malondialdehyde (MDA) ), and adhesion molecules (E-Selectin and ICAM-1). Quantification of IHC was performed according to the following semiquantitative scores[24] based on the percentage of positively stained

cells as following :0 , no staining ; 1,  $\leq 25\%$  ; 2 , 26-50% ; 3 , 51-75 % ; and 4 , 76-100% .

#### Statistical analysis

Data were collected, summarized, analyzed and presented using three statistical software programs: the statistical package for social science (SPSS version 22), Microsoft Office Excel 2013 and Med Calc 2014. Numeric variables were presented as mean and standard deviation. The results of Kolmogorov Smirnov test of normality distribution for numeric variables was significant and comparison of mean values among groups was carried out using Kruskal Wallis Test and then comparison between any two groups was done using Mann Whitney U test. Spearman correlation test was used to evaluate correlations between histological scores and immunohistochemical expression scores. P-value was considered significant when it was equal to or less than 0.05[25].

## RESULTS

### Effect of captopril on macroscopic features

Rectal instillation of acetic acid was applied in this study is one of the modality that has been used to produce macroscopical colonic mucosal injury in rats. Acetic acid triggered an intense inflammatory reaction on the 7<sup>th</sup> day of colitis induction , the distal colon showed severe macroscopic edematous inflammation . The colonic mucosa was inflamed, hyperemic and hemorrhagic compared to normal control group. However , oral administration of captopril and sulfasalazine after the induction of colitis significantly ( $p < 0.01$ ) attenuate the colonic damage scores as shown in table 2 and figure 1. On the other hand , captopril and sulfasalazine demonstrate significant ( $p < 0.01$ ) decrease in colonic weight comparable to the normal group as shown in table 2 and figure 2. Also , both captopril and sulfasalazine showed significant decrease ( $p < 0.01$ ) in DAI as shown in table 2 and figure 3. Furthermore both drugs elicit significant ( $P < 0.01$ ) decrease in macroscopic score as shown in table 2 and figure 4.

### Effect of captopril on histopathological features

The present study demonstrated characteristic histological features in untreated colitis, essentially loss of intestinal crypt architecture and sloughing of intestinal cells, reduced goblet cell number and presence of different inflammatory cell infiltration as demonstrated in figure 5 and 6. On the other hand both captopril and sulfasalazine treated groups significantly ( $P < 0.01$ ) attenuate histological features as judged by epithelization of colonic mucosa , reduction of edema and neutrophil infiltration as shown in figure 5. However captopril produced more significant ( $P < 0.05$ ) decrease in the pathological scores as compared with sulfasalazine , figure 5 and table 3.

### Effect of captopril on adhesion molecules (ICAM-1 and CD62)

The increased colonic ICAM-1 in the colitis group was found to be significantly ( $p < 0.05$ ) decreased after captopril and sulfasalazine treatment as shown in table (3) , figure 9 . However captopril produced more significant ( $P < 0.05$ ) decrease in the ICAM-1 level compared with sulfasalazine , figure 9. Also both tested drugs cause significant ( $p < 0.05$ )

decrease in CD62 compared to colitis group as shown in figure 11.

### Effect of captopril on proinflammatory cytokines (TNF- $\alpha$ and IL-6).

As shown in table (4) and figure 13 and 15 , colonic levels of TNF-  $\alpha$  and IL-6 showed drastic raise after acetic acid introduction compared to those of control group. In contrast these values were significantly ( $p < 0.01$ ) lower in rats treated with captopril and sulfasalazine . However , captopril elicit a more significant ( $p < 0.05$ ) decrease proinflammatory cytokines as compared with sulfasalazine treated group, figures 13 and 15.

### Effect of captopril on oxidative stress markers (MDA and MPO)

Administration of captopril or sulfasalazine to acetic acid treated rats significantly ( $p < 0.05$ ) reduced MDA compared to the colitis group as shown in figure 17 . On the other hand treatment with either captopril or sulfasalazine significantly ( $p < 0.01$ ) inhibited acetic acid induced MPO production in tissue as depicted in figure 19 .

## DISCUSSION

The present study showed that captopril significantly ( $p < 0.01$ ) reduced the area of colonic mucosal damage experimentally induced by acetic acid and the protective effect was comparable to sulfasalazine treated group . El-Medany *et al* [7] described protective effect for captopril against acetic acid induced colitis in rats and registered the nearly same observation of reduced area of mucosal damage in colon. Ariel *et al* [26] found that angiotensin converting enzyme inhibitors had significant role in reducing gross mice colonic mucosal damage following acetic acid induction, an observation that in accordance with the findings of the present study. Moreover captopril in present study showed significant reduction in colonic weight in comparison with the colitis group and this finding is correlated with Wengrower *et al*[27] study that showed colonic weight was significantly reduced in the group treated with the angiotensin receptor blocker (losartan) in comparison with the colitis group. Although, losartan is an angiotensin receptor blocker, and captopril is an angiotensin converting enzyme inhibitor, the similarity in colonic weight reduction might be mediated through a similar anti-inflammatory effect of both drugs (27, 28 ). Moreover in present study captopril and sulfasalazine significantly reduced DAI . This finding is comparable with observation of Mizushima *et al*[29] studied the effect of angiotensin receptor blockers on disease activity index (DIA) in experimentally induced colitis in mice; he found that DAI was significantly lower in candesartan-treated mice than in non -treated mice. Furthermore , similar to sulfasalazine ; captopril reduced macroscopic score of colon in experimentally induced colitis and this finding supported by observation of El-Medani *et al*[7].

In this work captopril had significantly reduced histopathological changes of colon in experimentally induced colitis. This finding in accordance with the finding of El-Medani *et al*[7] . The profound reduction in histopathological changes following the administration of captopril when compared to sulfasalazine may be attributed

to the more potent anti-inflammatory and anti-oxidant activity of captopril which have been proved in the current study as it was stated in previous sections.

The probable mechanism for the protective role of captopril against colitis may be due to the induced reduction of intercellular adhesion molecules in both endothelial cells and leukocytes via angiotensin receptor 1 (AT 1) mediated mechanism. This observation was made by (6, 29). From another point of view it has been proved that angiotensin receptors 1 (AT1) are expressed by inflammatory cells such as macrophages and the stimulation of these receptors will cause increase in genes transcription with their products which are well known pro-inflammatory mediators such as transforming growth factor -  $\beta$ 1 (TGF- $\beta$ 1) and tumor necrosis factor-  $\alpha$  (TNF- $\alpha$ ). So when the level of angiotensin I is reduced following administration of the angiotensin converting enzyme inhibitor (captopril), inflammation cascade will be reduced significantly (30,31,32)

### CONCLUSIONS

Captopril has a potent anti-inflammatory and anti-oxidant effects that can be used successfully in treatment of experimentally acetic acid induced colitis in rats.

### ACKNOWLEDGEMENTS

The author wishes to thank Dr. Eithar S Alkaragoli /College of dentistry /Al-Mustansiriya university for endless assistances during this research.

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